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(54) Title: MONOCLONAL ANTIBODIES AND THEIR USE

(57) Abstract

A monoclonal antibody to a penicillin-binding protein.

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MONOCLONAL ANTIBODIES AND THEIR USE

FIELD OF THE INVENTION

This invention relates to monoclonal antibodies and their use.

BACKGROUND OF THE INVENTION

In the late 1950's, Staphylococcus aureus caused considerable morbidity and mortality as a nosocomial pathogen due to its production of penicillinase. The introduction of penicillinase-resistant pencillins, such as methicillin, reduced the threat of these organisms in hospitals. However, within two years of the introduction of methicillin, resistant strains were isolated and epidemic outbreaks of methicillin-resistant S. aureus (MRSA) now occur throughout the world. The major problem is that MRSA is resistant to most of the commonly used anti-microbial agents including the latest generation of cephalosporins. Successful treatment requires treatment with vancomycin which, despite recent success in its purification, causes some toxic side-effects. Vancomycin resistance has not yet been reported, but the implications of such a situation are drastic at present, without the introduction of novel antibiotics.

The mechanism of methicillin resistance among S. aureus is not completely understood. However, determination of the mechanism has been aided by the discovery that resistant strains possess a penicillin-binding protein (PBP), designated herein as PBP-2' (but also known as PBP-2a), in addition to the four PBPs normally found in sensitive strains, i.e. methicillin-sensitive S. aureus (MSSA). PBP-2' has a low affinity for β-lactams; also its production is induced by the presence of these antibiotics. The occurrence of PBP-2a and of PBP-2' in MRSA is described by Rossi et al and by Ubukata et al, both in Antimicrobial Agents and

Chemotherapy, 27(5) (May 1985), respectively at 828-831 and 851-857.

Staphylococcus epidermidis is a major causative organism of prosthetic valve endocarditis. This organism also has a methicillin-resistant strain (MRSE).

Monoclonal antibodies are in general well known. They may be prepared by the process outlined by Koehler and Milstein, Eur. J. Immunol. 6 (1975) 292.

SUMMARY OF THE INVENTION

Monoclonal antibodies according to the present invention are to an "antigen" selected from penicillin-binding proteins, especially PBP-2', and to methicillin-resistant S. aureus or S. epidermidis.

The invention also comprises labelled monoclonal antibodies for use in diagnosing the presence of the antigens, each comprising a monoclonal antibody against one of the above-mentioned antigens or to a particular species thereof and having linked thereto an appropriate label. The label can be chosen from the group consisting of a radioactive isotope, enzyme, fluorescent compound, chemiluminescent compound, bioluminescent compound, ferromagnetic atom, or particle, or any other label.

The invention further comprises the process for diagnosing the presence of antigens as defined above in a specimen comprising contacting said specimen with the labelled monoclonal antibody in an appropriate immunoassay procedure.

Additionally, the invention is also directed to a therapeutic composition comprising a monoclonal antibody for the antigen and a carrier or diluent, as well as kits containing at least one labelled monoclonal antibody to the antigen.

DETAILED DESCRIPTION

The monoclonal antibodies of the present invention are prepared by fusing spleen cells, from a mammal which

has been immunised against the particular antigen, with an appropriate myeloma cell line, preferably NS0 (uncloned), P3NS1-Ag4/1, or Sp2/0 Ag14. The resultant product is then cultured in a standard HAT (hypoxanthine, aminopterin and thymidine) medium. Screening tests for the specific monoclonal antibodies are employed utilising immunoassay techniques which will be described below.

The immunised spleen cells may be derived from any mammal, such as primates, humans, rodents (i.e., mice, rats, rabbits), bovines, ovines and canines, but the present invention will be described in connection with mice. The mouse is first immunised by injection of the chosen antigen over a suitable period, e.g. of approximately eleven weeks. When the mouse shows sufficient antibody production against the antigen, as determined by conventional assay, it is given a booster injection of the appropriate antigen, and then killed so that the immunised spleen may be removed. The fusion can then be carried out utilising immunised spleen cells and an appropriate myeloma cell line.

The fused cells yielding an antibody which give a positive response to the presence of the particular antigen are removed and cloned utilising any of the standard methods. The monoclonal antibodies from the clones are then tested against standard antigens to determine their specificity for the particular antigen. The monoclonal antibody selected, which is specific for the particular antigen or species, is then bound to an appropriate label.

Amounts of antibody sufficient for labelling and subsequent commercial production are produced by the known techniques, such as by batch or continuous tissue culture or culture in vivo in mammals such as mice.

The monoclonal antibodies may be labelled with a multitude of different labels, such as enzymes,

fluorescent compounds, luminescent compounds, radioactive compounds, ferromagnetic labels, and the like. The present invention will be described with reference to the use of an enzyme labelled monoclonal antibody. Some of the enzymes utilised as labels are alkaline phosphatase, glucose oxidase, galactosidase, peroxidase, urease, and the like.

Such linkage with enzymes can be accomplished by any one of the conventional and known methods, such as the Staphylococcal Protein A method, the glutaraldehyde method, the benzoquinone method, or the periodate method.

Once the labelled monoclonal antibody is formed, testing is carried out employing one of a wide variety of conventional immunoassay methods. The particular method chosen will vary according to the monoclonal antibody and the label chosen. At the present time, enzyme immunoassays (EIA) are preferred due to their low cost, reagent stability, safety, sensitivity, and ease of procedure. One example is enzyme-linked immunosorbent assay (ELISA). ELISA is a solid phase assay system which is similar in design to the radiometric assay, but which utilises an enzyme in place of a radioactive isotope as the immunoglobulin marker. A dot blot assay for testing antibody reaction against small quantities of antigen has also been tested as a possible improvement of the ELISA assay.

Fluorescent-immunoassay is based on the labelling of antigen or antibody with fluorescent probes. A non-labelled antigen and a specific antibody are combined with identical fluorescently-labelled antigen. Both labelled and non-labelled antigen compete for antibody binding sites. The amount of labelled antigen bound to the antibody is dependent upon, and therefore a measurement of, the concentration of non-labelled antigen. Examples of this particular type of

fluorescentimmunoassay include heterogenous systems such as enzyme-linked fluorescent immunoassay, or homogeneous systems such as the substrate-labelled fluorescent immunoassay. The most suitable fluorescent probe, and the one most widely used, is fluorescein. While fluorescein can be subject to considerable interference from scattering, sensitivity can be increased by the use of a fluorometer optimised for the probe utilised in the particular assay and in which the effect of scattering can be minimised.

In fluorescence polarisation, a labelled sample is excited with polarised light and the degree of polarisation of the emitted light is measured. As the antigen binds to the antibody its rotation slows down and the degree of polarisation increases. Fluorescence polarisation is simple, quick, and precise. However, at the present time its sensitivity is limited to the micromole per litre range and upper nano-mole per litre range with respect to antigens in biological samples.

Luminescence is the emission of light by an atom or molecule as an electron is transferred to the ground state from a higher energy state. In both chemiluminescent and bioluminescent reactions, the free energy of a chemical reaction provides the energy required to produce an intermediate reaction or product in an electronically excited state. Subsequent decay back to the ground state is accompanied by emission of light. Bioluminescence is the name given to a special form of chemiluminescence found in biological systems, in which a catalytic protein or enzyme, such as luciferase, increases the efficiency of the luminescent reaction. The best known chemiluminescent substance is luminol.

A further aspect of the present invention is a therapeutic composition comprising one or more of the monoclonal antibodies to the particular antigen or

species, as well as a pharmacologically acceptable carrier or diluent. Such compositions can be used to treat humans and/or animals afflicted with some form of methicillin-resistant infections and they are used in amounts effective to cure; an amount which will vary widely dependent upon the individual being treated and the severity of the infection.

One or more of the monoclonal antibodies can be assembled into a diagnostic kit for use in diagnosing for the presence of the antigen.

In the past there have been difficulties in developing rapid kits because of undesirable cross-reactions of specimens such as urine with antiserum. The use of monoclonal antibodies can eliminate these problems and provide highly specific and rapid tests for diagnosis. For example, a kit can be used in pathology laboratories for the rapid detection of gram-negative bacteria in urine, or on an out-patient basis.

Additionally, conjugated or labelled monoclonal antibodies to the antigen and other gram-negative bacteria can be utilised in a kit to identify such antigens and organisms in blood samples taken from patients for the diagnosis of possible methicillin-resistance. The monoclonal test is an advance over existing procedures in that it is more accurate than existing tests; it gives "same day" results, provides convenience to the patient and improves therapy as a result of early, accurate diagnosis; and it reduces labour costs and laboratory time required for administration of the tests.

In addition to being sold individually, the kit could be included as a component in a comprehensive line of compatible immunoassay reagents sold to reference laboratories to detect the antigenic species.

One preferred embodiment of the present invention is a diagnostic kit comprising at least one labelled monoclonal antibody against a particular antigen or species, as well as any appropriate stains, counter-stains, or reagents. Further embodiments include kits containing at least one control sample of the antigen.

Monoclonal diagnostics which detect the presence of antigens can also be used in periodic testing of water sources, food supplies and food processing operations. Thus, while the present invention describes the use of the labelled monoclonal antibodies to determine the presence of a standard antigen, the invention can have many applications in diagnosing the presence of antigens by determining whether specimens such as urine, blood, stool, water and milk contain the particular antigen. More particularly, products of the invention could be utilised as a public health and safety diagnostic aid, whereby specimens such as water or food could be tested for possible contamination.

In order to obtain an antibody of the invention, it is necessary first of all to obtain the PBP, or the appropriate antigen. A selective screening procedure is required, both to estimate the different antibody responses in mice and to screen fusion products successfully. In the case of MRSA, both of these factors depend on the avoidance of interference by IgG-binding protein A present on the surface of certain MRSA (but not MRSE).

For MRSA, the antibodies which are produced must be of the IgM class or of an IgG subclass which can then be treated to avoid reaction with protein A. The antibodies used in the present invention may be of the subclasses IgG1 and IgG2a. The efficiency of antibodies may be ascertained at an early stage, by fluorescence microscopy, to determine whether the antibodies are

suitable for detection of whole organisms as well as differentiating between resistant and sensitive populations of cells.

Antibodies reacting with PBP-2' need to be purified and then treated in such a way as to use them directly for detection. This can be tested initially by blocking protein A with rabbit IgG using supernatants. Purified antibodies can be proteolytically treated with pepsin to produce $(Fab')_2$, the Fc portion being removed by passage through a protein A column; the antibody-binding portion can then be labelled for detection purposes, initially by fluorescence. Alternatively, antibodies could be selected for diminished protein A-binding by titration against protein A.

Assays may use purified antibodies or fragments thereof. If the antibodies detect whole organisms strongly by fluorescence, this indicates that the PBP-2' is surface-exposed on the cytoplasmic membrane. If an internal epitope of the antigen is all that was recognised, samples may be treated, e.g. with lysostaphin (5 min) to lyse the organisms prior to reaction with antibody.

The amount of PBP-2' expressed by MRSA in direct specimens can be low, owing to heteroresistance exhibited by these organisms. If insufficient PBP-2' is expressed, it may be necessary to cultivate the organism under favourable conditions (30 C, 5% NaCl, plus antibiotic). This would still remain a viable proposition if direct diagnosis were not possible. It should be possible to decrease the incubation period (at present optimal after 24-48 h) since visible growth may not be essential before PBP-2' expression is detectable using monoclonal antibody.

Different antigen types, e.g. whole MRSA cells, membranes isolated from such cells, or purified PBP-2',

may be used for immunisation. Purified PBP-2' for immunisation (and screening) can be obtained by electroelution from stained SDS-polyacrylamide gel; owing to the low affinity of the protein for penicillins, affinity purification, using columns with bound 6-aminopenicillanic acid, is of no use. Another problem has been the nature of the membrane protein and its dependence on the presence of the detergent (Genapol X100) which has been used to solubilise the PBP-2'; this detergent is compatible with FPLC technology. Ion exchange (Mono Q) and gel filtration (Superose 12) have been tested singly and in combination to give reasonable yields of PBP-2' and substantial purification. However, the need for detergent means that these steps do no necessarily remove other proteins of different molecular weight and charge. Therefore, after FPLC, further purification by ampicillin-affinity chromatography to remove PBP-2 and PBP-4 should yield a pure PBP-2'. Analysis of fractions can be fully characterised in a well-defined system (fluorography).

Hybridomas have been screened by EIA, e.g. ELISA in parallel against membranes from both resistant and sensitive organisms, and those producing a higher OD with the former antigen were selected for secondary screening both by EIA and by Western Blot against "resistant" membranes. Of 21 tested by this procedure, 12 showed a reaction with PBP-2' and 3 of these have been cloned. The 3 antibodies have been given the references 365/401, 332/423 and 332/452. Of these 365/401 reacts strongly against PBP-2' by Western Blot and weakly by EIA while the other two antibodies do not react with PBP-2' by Western Blot but do react strongly by EIA against extracts from resistant organisms. Immunoprecipitation experiments have revealed that 332/423 recognises

'native' but not denatured, PBP'2, thus explaining its reaction in ELISA.

332/423 is a good candidate for a rapid latex agglutination test. The antibody has been used successfully to affinity purify PBP-2'. For the purposes of latex agglutination, 332/423 may be bound covalently via its Fc region to protein A that has already been covalently bound to carboxylated polystyrene latex beads. This may restrict the non-specific binding of protein A present in antigen preparations.

Prior to commencing this project, membranes were tested with protein A-binding IgG antibodies in EIA. The results gave an indication that there was negligible protein A in the membrane preparation. However, EIA and the Western blots for the 21 McAbs indicated that some protein A was present. Thus, positive antibodies were found to light up both PBP-2' and protein A (approximately 45,000 daltons but not a homogeneous entity). This, in retrospect, provided the probable explanation as to why hybridomas showed reaction with "sensitive" membranes that was less than that with "resistant" membranes, i.e. a background reaction with protein A in both cases, supplemented with the reaction to PBP-2' present in only the "resistant" membranes.

The best antibody (by Western Blot) has been looked at by a sandwich microimmunofluorescence assay and it was found to pick out MRSA strongly and MSSA only very weakly. If cells were preincubated with rabbit IgG to block protein A, then the intensity of fluorescence staining was diminished; MRSA still stained up while no staining of MSSA was detected.

Two other clones have shown reaction with PBP-2' in MRSA samples.

The invention will be further illustrated in connection with the following Example which is set forth

for the purposes of illustration only and not by way of limitation.

The monoclonal antibodies of the present invention may be prepared generally according to the method of Kohler and Milstein, Eur. J. Immunol. 6 (1975) 292.

Example

A. Antigen Preparation:

MRSA cells (13134p-m+) were grown at 30°C in the presence of 5% NaCl and 10 mg/l methicillin. Membranes were prepared by lysostaphin digestion of peptidoglycan in intact cells and centrifugation (MRSA-membranes). MRSA-SDP was prepared from membranes by extraction with an equal volume of Triton X-100/NaCl at 37°C for 10 min. The supernatant, after high speed centrifugation, was used as antigen. Preparation of MSSA membranes and MSSA-SDP was identical except that isogenic cells (13136p+m-) were grown at 37°C without NaCl or antibiotic.

PBP-2' was purified from MRSA-SDP by SDS-PAGE (10% gels) and electroelution of the 75 kDa protein.

B. Assay

IgG2a antibodies were tested on MRSA membranes to check whether there was sufficient protein A in the antigen prpearation to allow non-specific immunoglobulin binding. Non-relevant McAbs did not react with MRSA membranes.

Testing tail bleeds by ELISA or by dot blot did not yield any information as there was no difference between MRSA and MSSA antigens due to antibody responses to the many shared common antigens. Screening hybridoma supernatants was successful at detecting antibody-producing cell lines but, again, there was a similar reaction of the antibodies with MSSA antigens. A combination of ELISA and subsequent Western blotting proved successful.

Initially in the ELISA, MRSA membranes and a goat anti-mouse alkaline phosphatase conjugate were used. Subsequently it was found that a better antigen was MRSA-SDP diluted in carbonate coating buffer and coated overnight at 4°C. At the same time it was necessary to use Bio-Rad goat anti-mouse HRPD as the conjugate and hydrogen peroxide/ MB for development. Western blots use the same conjugate and hydrogen peroxide/DAB plus nickel and cobalt development although this has been modified over the course of the project.

MRSA-SDP antigen does exhibit sticky characteristics that had been found already using MRSA-membranes; this is not a significant problem when stabilisation of characterised clones is being investigaged. The concentration of antigen (PBP-2') on ELISA plates coated with MRSA-SDP is low and this should be taken into account when assessing relevant McAbs. Analysis of supernatants from stabilisation was work done using a combination of ELISA and Western blots.

A dot blot assay for testing antibody reaction against small quantities of antigen from the clinical strains was tested as a possible improvement to the ELISA assay. Triton/NaCl-extracted antigen (2 µl) was applied to nitrocellulose and, after incubation with antibody, reactions were visualised using a secondary goat anti-mouse alkaline phosphatase with Vectastain black substrate.

C. Immunisation

The following immunisation schedule has proved successful: day 0, formalinised MRSA cell + FCA, i.p.; day 30, PBP-2' + FIA,i.l.; day 44, PBP-2' i.v.; day 48, splenocytes fused.

D. Fusion/Antibody Selection

Mice for fusion were selected on the basis of results obtained by Western blot of tail bleeds tested

against PBP-2'. Mice with reactions against PBP-2' were selected (Immunisation 256, Mouse No. 1 - fusion 332, Mouse No. 2 - fusion 365).

1. Hydridomas were screened primarily against MRSA membranes and against MSSA membranes by EIA. Those reacting strongest against the former were tested by Western blot against MRSA membranes. 332/236 reacted strongly against PBP-2', others reacted weakly with PBP-2' (332/360, 332/452).
2. Subsequently, hybridomas were screened against MRSA-SDP by EIA and then positives screened by Western blot against MRSA-SDP, MSSA-SDP and PBP-2'. One McAb (365/401) was selected that reacted against PBP-2'.

Selected antibodies being evaluated are 332/423, 332/452 and 365/401.

E. Antibody Characterisation

365/401 detects PBP-2' and the same antigen in MRSA membranes by Western blot. The cell line has been cloned. The original subclass was mixed (IgG1/IgM) but all three clones are of the IgG1 subclass.

MRSA-SDP and MSSA-SDP were subjected to IEF on an agarose gel (pI range 3 to 10). A contact blot was made which was then probed with 365/401.43 at 1:5 dilution. A diffuse spot was apparent in the MRSA-SDP sample only.

It has been confirmed that detergent extracts of membranes contain protein A (ca. 45 to 50 kDa). 332/423.10.P1D, an IgG2a monoclonal, does not react with PBP-2' in Western blots but reacts with protein A in the sensitive and resistant isolates of 13136 strain. Protein A was confirmed in these strains using a chicken anti-protein A serum. A certain amount of blocking could be achieved by incubating antibody in the presence of normal mouse serum. 365/401.43.P11 and 352/452.19 P1D only reacted weakly with protein A, indicative of their

Ig G1 subclass. Many of the clinical strains were found to possess protein A when tested with the anti-protein A serum in a dot blot system.

E. Antibody Purification

Antibodies 332/401 and 332/352 were purified by the Protein A-Sepharore method. Antibody 332/423 was purified by SP-Sephadex, ammonium sulphate, DEAE-Sephacell.

F. Antibody Conjugation

Because the alkaline phosphatase conjugate 365/401.43.P1I.AP1 had a much lower ELISA titre than the HRP conjugate, 365/401.43.P1I.HRP1, it has been necessary to do comparative Western blots which have provided extra information on this assay system:

a) 332/423.10 and 332/452.19 (purified antibodies tested at approximately 20 µg/ml and 2 µg/ml. The former antibody, especially, shows non-specific binding to a number of S. aureus antigens. There is apparently weak binding of both to PBP-2' that is no greater when antibody is absent. The major binding is to an antigen having a molecular mass of 48 kDa (one band on MRSA-SDP, two considerably stronger with MSSA-SDP). The IgG2b antibody (332/423.10) shows a much stronger reaction with what could be protein A, present in greater amounts in the sensitive strain; 332/452.19 is an IgG1 McAb.

b) 365/401.43.P1I and its three conjugates all exhibit weak non-specific reaction with 45-50K proteins in MRSA and MSSA but show a strong reaction to PBP-2' (78 kDa) in MRSA-SDP only. A direct comparison of the reactions of conjugates and purified antibody is tabulated below. In conclusion, using secondary alkaline phosphatase conjugate with Vestastain black or the AP1 conjugate with the same substrate was

more effective that HRP conjugates with hydrogen peroxide and UAB/nickel/cobalt. Appropriate working dilutions would be 1:2,000 (365/401.43.P1I), 1:400 (365/401.43.P1I.AP1) and 1:400 (365/401.43.P1I.HRP1 and HRP2). Higher concentrations of conjugates result in increased background stain.

Table 1:

Reaction of 365/401.43 P1I and its conjugates with PBP-2' by Western blot

Primary antibody with dilution		Secondary antibody with dilution		Chromogen	Reaction PBP-2'	Background stain
P1I	1:100	G anti-M:AP	1:1,000	Vectastain	4+	-
	1:1,000	G anti-M:AP	1:1,000	Vectastain	4+	-
	1:1,000	G anti-M:HRP	1:3,000	DAB	3+	+/-
	1:2,000	G anti-M:HRP	1:3,000	DAB	3+	+/-
		P1I.AP1	1:20	Vectastain	2+	3+
		P1I.AP1	1:200	Vectastain	3+	1+
		P1I.AP1	1:1,000	Vectastain	2+	-
		P1I.HRP1	1:200	DAB	2+	1+
		P1I.HRP1	1:1,000	DAB	1+	-
		P1I.HRP1	1:2,000	DAB	1+	-
		P1I.HRP2	1:200	DAB	2+	1+
		P1I.HRP2	1:1,000	DAB	1+	-
		P1I.HRP2	1;2,000	DAB	1+	-

G. Functional Evaluation

365/401.43.P1I was coupled to Sepharose via CNBr and mixed with MRSA-SDP to separate out native PBP-2' by affinity chromatography. Using different conditions of pH, no detectable PBP-2' was bound to the matrix. When 'denatured' PBP-2' was added, batchwise, to the matrix a small proportion of PBP-2' did bind to the antibody. Approximately 80% of the antibody was bound to the Sepharose so it would appear that either the antibody only recognises a denatured epitope on PBP-2' or the antigen binding site of the antibody has been obscured by covalent linkage to Sepharose. Alternatively, since the antibody recognises 'native' PBP-2' by ELISA it may be that the antibody has too low an affinity for antigen purification. An alternative to 365/401 is to evaluate the potential of 332/423.10 which has a higher titre on ELISA and can be detected using goat anti-mouse:AP secondary conjugate. In this case the antibody does not recognise PBP-2' in the Western blot assay.

Immunoprecipitation experiments have revealed that 332/423.10 recognised 'native' but not denatured PBP-2', thus explaining its reaction in ELISA. Two immunoprecipitation experiments were done to examine whether the latter antibody recognises 'native' PBP-2'. In the first, antibody and MRSA-SDP were incubated and immune complexes precipated by addition of S. aureus strain Cowan 1 cells (which possess protein A) to bind the Fc portion of the IgG2a McAb. The precipitate was solubilised, separated by SDS-PAGE and a Western blot probed using 365/401.43 to detect any PBP-2' bound to the cells. While PBP-2' was detected, a control antibody (318/305.33, anti-Campylobacter McAb) also appeared to bind some antigen. The latter resulted from being unable to properly wash the cells in the experimental protocol used. Because of this, a second

experiment was done using protein A-Sepharose to bind immune complexes. In this case, 332/423.10 but not irrelevant McAb 318/305.33 precipitated PBP-2' present in MRSA-SDP which was detected using 365/401.43.P1I.HRP1. Using this conjugate enabled the detection of PBP-2' in the absence of heavy and light immunoglobulin chains detectable with a secondary anti-mouse conjugate in the first experiment.

Two antibodies have been evaluated against 21 clinical isolates. The results tabulated below indicate that 332/423.10 recognises all 12 resistant strains but none of the 9 sensitive strains by ELISA and that 365/401.43.P1I.AP1 reacts with PBP-2' present on only the resistant strains by Western blot. The latter antibody is not functional on ELISA or dot blot systems while the former antibody does not recognise PBP-2' in the Western blot system.

Table 2 Resistant Staphyloccocus aureus strains (a)

Strain	Batch No.	MIC value (b) (mg/l)	EL
21/88 (6769, Paris)	3253	32	0
15/25 (5152, London)	3252	>128	0
21/82 (Hornsby, Aust)	3333	>128	0
13136 p-m+ (Lacey)	3317	128	0
18/43 (USA)	3311	>128	0
13136 p+m+ (Lacey)	3339	>128	0
15/45 (Lemon, 1960)	3315	32	0
18/39 (USA)	3314	>128	0
15/41 (Gray, 7705)	3248	64	0
21/86 (JGD, Japan)	3249	32	0
17/35 (Yarwood)	3251	128	0
16/43 (Cheslyn, 811R)	3337	64	0

(a) Western blot results with 365/401.43.P1I AP1 at 1,5000 showed the presence of PBP-2' in all 12 resistant strains (not quantitative).

(b) Minimum inhibitory concentrations (MIC) of methicillin determined on Mueller-Hunton agar containing 5% NaCl with an inoculum of 10C4 cfu and incubated at 37°C for 18 h.

(c) ELISA results expressed as the OD obtained using an antigen concentration of 1:2 (Triton/NaCl extract) and neat 332/423.10.361 tissue culture supernatant fluid using goat anti-mouse:HRPO and TMB/Hydrogen peroxide for development (20 minutes).

Table 3 Sensitive Staphyloccocus aureus strains (a)

Strain	Batch No.	MIL value (b) (mg/l)
10/31 (3414 Coote)	3335	8
21/49 (7919 W)	3318	4
13136 p-m- (Lacey)	3308	4
21/31 (8108)	3312	2
21/34 (7842)	3309	4
17/47 (1834)	3310	8
21/54 (7950)	3307	2
21/22 (Baldock)	3250	4
20/32 (Berry)	3316	8

(a) Western blot results with 365/401.43.P1I AP1 (1:500) negative for all strains and ELISA results with 332/423.10.361 (see Table 2, footnote c) all negative.

(b) See Table 2 footnote (b).

In addition 365/401.43 has been found to react with the PBP-2' that is produced by a sensitive strain of S. aureus carrying a In 551 trasnposan insertion (Berger-Bachi et al (1986), Eur.J.Clin. Microbiology 5:S97-701).

An important experiment (related to the immunoprecipitation experiment above, adapted from the method of Schneider et al, J.Biol.Chem (1982) 257: 10766-69) has enabled us to affinity purify PBP-2' from a crude extract using 332/423.10.361.P10. The antibody (5 mg) was bound to protein A-Sepharose (1 ml) followed by cross-linking of the complex with dimethyl suberimidate to allow optimal spatial orientation of the antibody on

the matrix. PBP-2' bound to the column and after several washes could be eluted with 0.05M diethylamine (pH 11.5) containing 0.05% sodium deoxycholate. Clean PBP-2' was detected by silver staining and Coomassie staining of gels and by using 365/401.43.P1I.AP2 as a probe or blots.

CLAIMS

1. A monoclonal antibody to a penicillin-binding protein.

2. A monoclonal antibody to a penicillin-binding protein in a strain of Staphylococcus.

3. A monoclonal antibody to PBP-2' in a methicillin-resistant strain of Staphylococcus.

4. A monoclonal antibody to methicillin-resistant Staphylococcus aureus.

5. A monoclonal antibody to methicillin-resistant Staphylococcus epidermidis.

6. A monoclonal antibody according to any preceding claim, which is labelled.

7. A monoclonal antibody according to claim 6, wherein the label is a radioactive isotope, enzyme, fluorescent compound, bio-luminescent compound, chemi-luminescent compound, or ferromagnetic atom or particle.

8. A monoclonal antibody according to claim 7, wherein the label is an enzyme capable of being used in an enzyme-linked immunoassay procedure, a fluorescent compound or probe capable of being used in an immuno-fluorescent, fluorescent, enzyme-fluorescent, fluorescence-polarisation or photon-counting immunoassay procedure, a chemi-luminescent compound capable of being used in a luminescent or enzyme-linked immunoassay procedure, or a bio-luminescent compound capable of being used in a bio-luminescent immunoassay procedure.

9. A monoclonal antibody according to claim 8, wherein the label is an enzyme selected from alkaline phosphatase, glucose oxidase, galactosidase and peroxidase, fluorescein, a chemi-luminescent compound selected from luminol and luminol derivatives, or a bio-luminescent compound selected from luciferase and luciferase derivatives.

10. A monoclonal antibody according to any preceding claim, for use in treating Staphylococcus infections.

11. A process for diagnosing the presence of an antigen of Staphylococcus in a specimen, which comprises contacting the specimen with a monoclonal antibody according according to any of claims 6 to 9 in an immunoassay procedure appropriate to the label.

12. A therapeutic composition which comprises a monoclonal antibody according to any of claims 1 to 10 and a pharmaceutically-acceptable carrier or diluent.

13. A kit for use in diagnosing the presence of a staphylococcal bacterial infection, which comprises a monoclonal antibody according to any of claims 1 to 10 and, as a control, a known Staphylococcus antigen.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 87/00635

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 12 P 21/00; G 01 N 33/569; G 01 N 33/577; A 61 K 39/40; //(C 12 P 21/00; C 12 R 1:91)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	G 01 N; C 12 P; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	WO, A, 86/02358 (TECHNOLOGY LICENCE CO. LTD) 24 April 1986 see the whole document --	1-13
Y	Biological Abstracts, volume 83, no. 3, 1987, (Philadelphia, PA., US), R. Hakenbeck: "Penicillin-binding proteins of penicillin-susceptible and penicillin-resistant pneumococci: Immunological relatedness of altered proteins and changes in peptides carrying the β-lactam binding site", see page AB-171, abstract 21442, & Antimicrob Agents Chemother 30(4): 553-558, 1986 --	1-13
Y	Biological Abstracts, volume 82, no. 6, 1986, (Philadelphia, PA., US), R. Hakenbeck et al.: "Antibodies against the benzylpenicilloyl moiety as a probe for penicillin-binding proteins", see page AB 860, abstract 57443, & Eur J Biochem ./.	1-13

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
4th December 1987	10 FEB 1988
International Searching Authority: EUROPEAN PATENT OFFICE	Signature of Authorized Officer: P.C.G. VAN DER PUTTEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	157(1) 101-106, 1986 --	
A	Biological Abstracts, volume 65, 1 January 1978, (Philadelphia, PA., US), C.E. Buchanan et al.: "Antibody to the D-alanine carboxypeptidase of Bacillus subtilis does not cross-react with other penicillin-binding proteins", see page 411, abstract 4264, & J. Bacteriol 131(3): 1008-1010, 1977	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 8700635 SA 18567

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 28/01/88
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8602358	24-04-86	EP-A- 0203088	03-12-86